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Dermatophyte infections are being studied in germfree and conventional rats and guinea pigs and nude mice. The rat is a good model for dermatophyte infections. The primary infection is not as severe as in guinea pigs. An erythema-like reaction (with hyphae in the skin) clears in the germfree rat in 13-14 days and the hair grows back. Conventional rats manifested signs of low grade infection, no hair regrowth for 60-70 days. The germfree rat does not manifest infection with E. floccosum or M. canis. (cont on p1473B)

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Comparison of infection in germfree and conventional guinea pigs indicates that a skin infection does occur in the germfree guinea pigs but the primary course of infection is twice as long as in the conventional guinea pigs. No colonization of the GI tract of germfree guinea pigs was observed.

1473 B

A

Acquired Immunity to Pathogenic Fungi

Annual Progress Report

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Edward Balish, Ph.D.

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Progress Report

Introduction

Germ-free animals are a unique animal model for studies on infectivity, pathogenesis, acquired immunity, prophylaxis, and therapy of dermatophyte infections because: 1) there are no skin or gut bacteria to augment (or hinder) the true course of an experimental dermatophyte infection, 2) germ-free animals have not had any previous exposure to viable dermatophytes and thus the problem of contending with immunological effects from prior subclinical fungal infections is eliminated, 3) true immune responses (both antibody, AMI, and cell mediated immunity, CMI) can be assessed without competition and/or antigenic stimulation by the viable bacteria and other fungi that are so prevalent in, and on, experimental animals, 4) when activated, the antibody and cell mediated immune responses of the germ-free animal are every bit as good as, and in some instances better than, the conventional animal. Therefore, an assessment of pure CMI and AMI responses to a viable dermatophyte infection can be made, 5) true effect of therapeutic agents (either topical or systemic, i.e. steroids or Griseofulvin) on the infectious disease caused by the dermatophyte can be assessed without interference from competing bacteria, or previous subclinical dermatophyte infections, 6) the effect of skin bacteria on the course of dermatophyte infections can be evaluated in the gnotobiotic model.

It is now known that T. mentagrophytes and other dermatophytes, when looked for, can be isolated from all conventional guinea pig, rat, and mouse colonies. This limits the usefulness of conventional guinea pigs, rats, and mice in studies on immunity, prophylaxis and therapy of dermatophyte infections.

Our research program is using germ-free rats and guinea pigs as models for dermatophyte infections. We will also, if our breeding program works out, be able to infect the germ-free beagle dog. We have infected conventional rats, guinea pigs and flora defined nude mice (i.e. an animal without a functional T-cell capability) with dermatophytes. Our results with each of these animal models is detailed below.

Microorganisms: Most of our studies have been carried out with Trichophyton mentagrophytes; however, we have also used Microsporum canis, Epidermophyton floccosum and C. albicans in some of our studies.

General Comments

Studies with germ-free and conventional rats: Can dermatophytes, by themselves, infect animals that are free of a viable bacterial and viral flora? To date our results indicate that germ-free rats (and guinea pigs; see below) can be infected by a pure culture of dermatophyte. Obvious infectious lesions, were observed in germ-free and conventional rats, (and guinea pigs; see below) challenged with T. mentagrophytes. We were unable to cause any obvious dermatophyte lesions with Microsporum canis or with Epidermophyton floccosum in the germ-free rats. It is also worth noting that none of the 3 fungi used i.e. E. floccosum, M. canis, or T. mentagrophytes was able to colonize the bacteria free GI tract, oral cavity, nasal cavity or lungs of the germ-free rat: only T. mentagrophytes was found in low numbers

(10^2-10^4 gm of feces from cecum and colon only). This is most interesting because even in the absence of competing bacteria, these fungi do not colonize or invade mucosal epithelial cells or keratinized stomach epithelium. Conversely, the germ-free rat does show invasive hyphae in the secretory portion of the stomach after monoassociation with C. albicans.

Another interesting aspect of the germ-free rat model is that T. mentagrophytes sets up a visible infection (hyphae invade epidermis) on the skin at the inoculated site. None of the germ-free rats showed any lesions other than at the site of inoculation. No fungal overgrowth occurred in the chambers or on the animals skin at sites other than those experimentally infected. The T. mentagrophytes lesion (a red erythema) in germ-free rats clears in 14 days. The lesion is not as severe as we observed in the guinea pig and it clears sooner in the germ-free rat(14 days) and hair grows back by 30 days. Converse-ly, conventional rats had scaly skin and no hair growth occurred at the inoculated site for 60-70 days.

The chronic T. mentagrophytes infection that persisted for 60-70 days in conventional rats is of interest because it was a low grade infection and not an ulcerating lesion like we see in guinea pigs. This conventional rat model could be very useful for trials in topical or systemic therapy of fungal infections. The only visible evidence of dermatophyte infection was scaly skin and a lack of hair growth for 60-70 days.

To date our studies have shown that skin from T. mentagrophytes lesion sites, in the conventional rat only, have a histopathological picture similar to psoriasis. This is a most interesting aspect of the work and it may indicate that a dermatophyte infection may be a mechanism for triggering psoriasis. However, since it did not occur in the germ-free state it may indicate that skin bacteria are involved in its etiology. These experiments on rats also demonstrate that conventional rats can carry T. mentagrophytes on their skin, without overt indications of dermatophyte infection, for prolonged time periods; culture and histology and no growth of hair are the only indications of gross abnormality to the rats skin.

It should be remembered that in Viet Nam, rats appeared to be an important vector for T. mentagrophytes. We have shown that rats have the capacity to carry T. mentagrophytes subclinically on the skin and in the GI tract for at least 70 days (termination of our experiment).

A significant stimulation of peyers patches was also observed in the small intestine of germ-free rats infected with T. mentagrophytes. No fungi could be cultured or demonstrated with histological sections of peyers patches. There may have been some fungal products consumed by the rats that accounted for the stimulation of peyers patches.

Antibody and Cell Mediated Immune Responses in the Dermatophyte Infected Germ-free Rat:

AMI -- We have observed that germ-free rats manifest a poor immunoglobulin response against the invading dermatophyte. A primary and secondary challenge of germ-free rats with T. mentagrophytes resulted in an increased level of immunoglobulins in only 1 of 6 bacteria-free animals. Immunoelectro-

phoresis demonstrated no great increase in gammaglobulins within 70 days after challenge. We were only able to demonstrate 1 out of 6 rats showing a positive precipitin test to purified trichophytin, crude cell wall antigen or soluble cytoplasmic antigen. We are currently assessing the capacity of serum from germ-free, T. mentagrophytes monoassociated, and conventional rats to inhibit T. mentagrophytes in agar diffusion studies. Initial results indicate, that serum from the monoassociated rats is just as inhibitory as serum from germ-free and conventional rats. Further work on these sera indicate that the inhibition of T. mentagrophytes by serum appears not to be associated with specific immunoglobulin. A student is starting to purify rat serum proteins for further clarification of this serum inhibition of dermatophytes. He will use serum from germfree,monoassociated and conventional rats

CMI -- In vitro blastogenesis of splenic lymphocytes against phytohemogglutin (PHA) and conconavolin A (Con A) are poor in the dermatophyte infected bacteria-free rats. However, at the same time intervals, the infected rats splenic lymphocytes appear to acquire a good capacity (10 fold) to respond against the T. mentagrophytes antigens we used (crude autoclaved extract, formalinized spores, purified T. mentagrophytes antigen from Dr. Jones). Lymphocyte blastogenesis (at various time periods after infection and clearing of the lesion) indicates to us that clearance of a dermatophyte infection appears to be associated with the acquisition of a CMI response in the rat. It should also be pointed out that the skin testing of conventional rats that have the chronic infection is very positive with purified T. mentagrophytes antigen, formalinized spores, and crude autoclaved antigen. We do not see, however, a typical delayed type hypersensitivity response in the monoassociated germ-free rat. A delayed basophil response is very prominent in the conventional rat and this may indicate that a Jones-mote type of reaction (cutaneous Basophil hypersensitivity) is taking place rather than the typical pure monocyte response as seen in the classic delayed type hypersensitivity response (to PPD) in tuberculin positive individuals.

Summary of Rat Experiments: This is a good model; a) the primary infection is not as severe as in guinea pigs. The erythema like reaction (with hyphae in skin) clears in the germ-free rat in 13-14 days and the hair grows back. The hair does not grow back in conventional rats and a low grade persistent fungal reaction can be seen on the skin for 60-70 dyas. There also appears to be a parakeratosis associated with the cleared infected site and it is very similar to psoriasis. The germ-free rat does not become overgrown with the dermatophytes used (T. mentagrophytes, E. floccosum, M. canis). However, of the 3 dermatophytes used only T. mentagrophytes caused any obvious dermatophyte-like pathology in the rat model. E. floccosum induced a brown pigmentation (hyperkeratosis) over the inoculated site but no obvious fungal type of lesion. However, the latter pathology (hyperkeratosis) was also observed in the skin of male but not female rats. Neither E. floccosum or M. canis survived in the germ-free environment. They appeared to die out after several challenges of the germ-free animal. This may indicate that bacterial associations are needed for the survival of the latter 2 agents on conventional rats. (We will be looking into this aspect of dermatophyte infections in the near future.)

Nude Mouse Data: The nude mouse is an animal that is congenitally athymic; therefore, it lacks the capacity for the T-cell (CMI) arm of immunity. If dermatophyte infections are controlled by T-cell dependent immunity then

the nude mouse should not be able to control dermatophyte infections. We were, however, not able to induce dermatophyte lesions on the skin of nude mice even though skin cultures were positive 7 days after challenge. Our procedure used a spore inoculum (100,1000,10,000 spores per occluded site). We were not able to induce any lesions on the skin of the nude mice (~7 nudes used in experiments; similar inoculum took very well on the conventional rat and the guinea pig).

The nude mouse was further evaluated by I.V. injection of C. albicans. Our studies demonstrated that the nude mouse cleared an I.V. C. albicans challenge better than their littermates (i.e. same strain of mice but with a functional thymus). C. albicans infections are thought to provide classic examples of the importance of T-cell function in host resistance to fungal disease. Our nude mouse data indicates that the importance of T-cell function in immunity to dermatophyte and candida infections is not as clear cut as one would gather from the existing literature. Our initial results on the nude mouse indicate other host immune factors are operating to control fungal disease.

The flora-defined nude mouse did not get obvious dermatophyte lesions. We plan to have germ-free nudes in the near future and we will try to infect them with dermatophytes. We have also challenged thymus reconstructed nude mice with C. albicans (I.V.). We found the latter nudes (with proven T-cell function after thymic implants) were just as susceptable to an (I.V.) C. albican challenge as the normal mice. It appears that the thymus may have suppressed immunity to systemic candidiasis. We are going to infect thymus implanted nudes to see if they can also now get a dermatophyte lesion on the skin.

Germ-free and Conventional Guinea Pigs: Our infections of conventional guinea pigs (Hartley strain) have followed the pattern of dermatophyte infection already described by the LAIR group (Akers, Kerbs, Jones, et al) for conventional guinea pigs. Our only information to add to the conventional system is in the area of the cellular response (histology) of lesions and skin tests. We are seeing much more Eosinophil and Neutrophil involvement in lesions and skin tests on conventional guinea pigs than one would normally expect in a classical PPD delayed type hypersensitivity response.

We have also quantitated the increase in fungi per sq. cm of skin in infected conventional guinea pigs. The counts 25-50 colony forming units per cm^2 of skin at day 0 increase to 10^5 CFU/cm^2 on day 7, and drop to 10^4 CFU/cm^2 on day 14; less than 50 CFU/cm^2 of skin are present on day 21. We have also shown that in vitro blastogenesis of guinea pig lymphocytes (soluble and particulate T. mentagrophytes antigen) increases on about day 7 and 14 and seems to correlate with the time the fungi are being cleared from the skin of the guinea pigs. The delayed hypersensitivity skin test response of conventional guinea pigs is heightened on day 14 and 21. Apparently in vivo and in vitro correlates of cell mediated immunity coincide with clearance of fungi from infected skin.

Germ-free Guinea Pigs: We have now for the first time infected germ-free guinea pigs with T. mentagrophytes. The overall infection rate was 100%. The infection and host response seems to be more severe and persistent (no regrowth of hair and still obvious, open, serous lesions at 32

days past infection). The infection has not spread beyond the original site of inoculation and no obvious fungal overgrowth occurs in the germ-free isolator. We plan to allow the infected animals to continue on in order to see how long it takes for the lesion to clear and hair to grow back in the monoassosicated(gnotobiotic) state. Now that we have established breeding colonies of conventional guinea pigs and have acquired experience in breeding and caesarian delivery of guinea pigs we will be able to infect more germ-free guinea pigs to pursue our goals for this research program.

This germ-free model will be an excellent one for studying therapy and prophylaxis because of its chronic persistent nature. Also, it is very obvious that prior immunological experiences or skin bacteria of conventional guinea pigs does reduce the severity of dermatophyte infections in the conventional state. The lesions on conventional guinea pigs are not as severe or as prolonged as we have observed in the germ-free guinea pig model.

Studies on skin tests, blastogenesis, serum responses (i.e. antibody production) are now and will continue to be in progress on the germ-free guinea pigs when the initial observations on infectivity, primary and secondary, are complete.

We have had experience with blastogenesis and skin tests in conventional guinea pigs. We are seeing increased blastogenesis in days 7 and 14 after infection. This corresponds with a decrease in fungi in the skin and also with the onset of delayed type hypersensitivity. However, histology of the skin test sites reveals a heavy infiltration of Neutrophils and Eosinophils rather than monocytes as we would expect.

Germ-free Dogs: We should be able to infect germ-free dogs (beagles) with dermatophytes in the next 3-6 months. Our program of raising germ-free dogs is now to the point where we are getting healthy animals and we should be able to derive and raise the germ-free beagles (6--3 males, 3 females) for our dermatophyte study.

Humidity: In our studies on rats we tried increasing the humidity inside of plastic isolators to see if this would exacerbate the fungal infections (T. mentagrophytes, M. canis, E. floccosum). Pans of water were put into the isolator, airflow was slowed down and the resultant increased humidity did not appear to cause any obvious increase in the severity of the T. mentagrophytes infection or activate the M. canis or E. floccosum challenged animals. We will try to assess this reaction in guinea pigs when we get more animlas in the germ-free state and monoassociated with T. mentagrophytes.

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